JOURNAL OF BACTERIOLOGY, July 1995, p. 4131–4133 Vol. 177, No. 14
0021-9193/95/$04.00+0
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NOTES

Induction of Cold-Responsive Proteins in *Vibrio vulnificus*

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Received 26 August 1994/Accepted 4 May 1995

We have studied the response of *Vibrio vulnificus* to temperature shifts (23 to 13 C) within the organism's permissive growth range. Cold shift induced a diminution in protein synthesis. Following a short lag, cells began growth at a new rate. Forty proteins were induced by this downshift.

The survival of bacteria depends on their ability to respond to environmental change. For estuarine bacteria, the exposure to changes in temperature is a constant stress. Culture-based surveys of the aquatic environment show that fluctuation in the bacterial population occurs throughout the year; a species prevalent in the summer, for example, may not be found in surveys taken during the colder months. Studies with *Escherichia coli* have begun to define the effects of temperature shifts on bacterial physiology (for a review, see reference 3). Transferring *E. coli* from culture at 37 to 10°C induces a growth lag, followed by physiological changes allowing the cell to continue growth at the new temperature (5). Studies with *E. coli* have also defined the cold shock response, a specific pattern of gene expression and protein synthesis which accompanies changes in growth temperature (2–4, 12). Proteins involved in the *E. coli* cold shock response participate in central cellular control mechanisms, including DNA supercoiling, recombination, transcription, and translation. The major *E. coli* cold shock protein, CspA (1), may be a low-temperature transcriptional activator (5). Although homologs of this protein have been identified in the psychrophilic bacterium *Arthrobacter protophormiae* (11), *Pseudomonas fluorescens* (11), and *Bacillus subtilis* (13), how the responses of laboratory *E. coli* strains reflect those of bacteria in nature is not known.

We have been studying the response of the bacterium *Vibrio vulnificus* to environmental change to better understand this organism's survival strategies in nature. *V. vulnificus* (for reviews, see references 7 and 8) is prevalent in the estuarine waters of the Atlantic Ocean and the Gulf of Mexico. This organism is capable of living free in the water and within oysters and other filter-feeding mollusks. Its presence in edible mollusks contributes to its position as a formidable human pathogen.

This report characterizes the response of *V. vulnificus* to a moderate temperature downshift, from 23 to 13°C. Following a shift to 13°C, *V. vulnificus* experiences a decline in protein synthesis but continues to grow, albeit at a reduced rate. Exposing *V. vulnificus* to more dramatic temperature shifts induces a state in which the organism is still viable (8) but in which growth and protein synthesis are both stopped, making studies of lower temperatures complicated. Characterizing the factors that contribute to this bacterium's response to temperature downshifts may provide a gateway to understanding the survival strategies of bacteria in the environment.

The temperature range allowing growth of *V. vulnificus* C7184 (opaque) in our defined medium (6) is 10 to 42°C (data not shown). Cells growing at 23°C doubled every 3.0 h. Following a shift to 13°C, the generation time increased to 13.1 h. Protein synthesis rates for mid-log-phase cultures at room temperature were compared with those for cultures subjected to a shift to 13°C. Unlike the pattern detected with *E. coli* (3), the shift to a new growth rate of *V. vulnificus* was accompanied by little lag. This may reflect an adaptation of this marine species to a natural pattern of wide temperature variation.

The rate of protein synthesis following temperature downshift, measured after trichloroacetic acid precipitation, showed an immediate and sharp decline (Fig. 1). Cells were cold shocked and pulse-labeled with [^{35}S]methionine. Radiolabeled proteins were separated by two-dimensional gel electrophoresis by the modification by Pederson et al. (10) of O'Farrell's method and then quantitated by densitometry. Forty proteins were synthesized at higher levels during the cold stress response (Fig. 2). Five of these reached peaks of expression within 15 min of cold stress. Ten more were maximally expressed by the 30-min time point. Five were maximally expressed at 1 h. The greatest number of proteins, 13, peaked after 2 h. Seven peaked after 4 h.

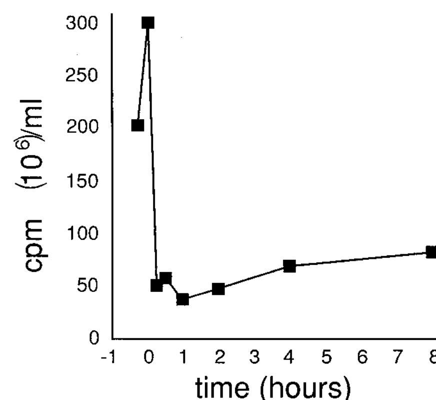

FIG. 1. Protein synthesis following temperature shift. Following cold shift, aliquots of cells were pulse-labeled for 10 min with [^{35}S]methionine and then chased for 3 min with an excess of cold methionine. Cells were then harvested, quick-frozen, and lysed. A fraction of the lysate was trichloroacetic acid precipitated, and incorporation of label into protein was measured. Preshift points are values for log growth at 23°C.

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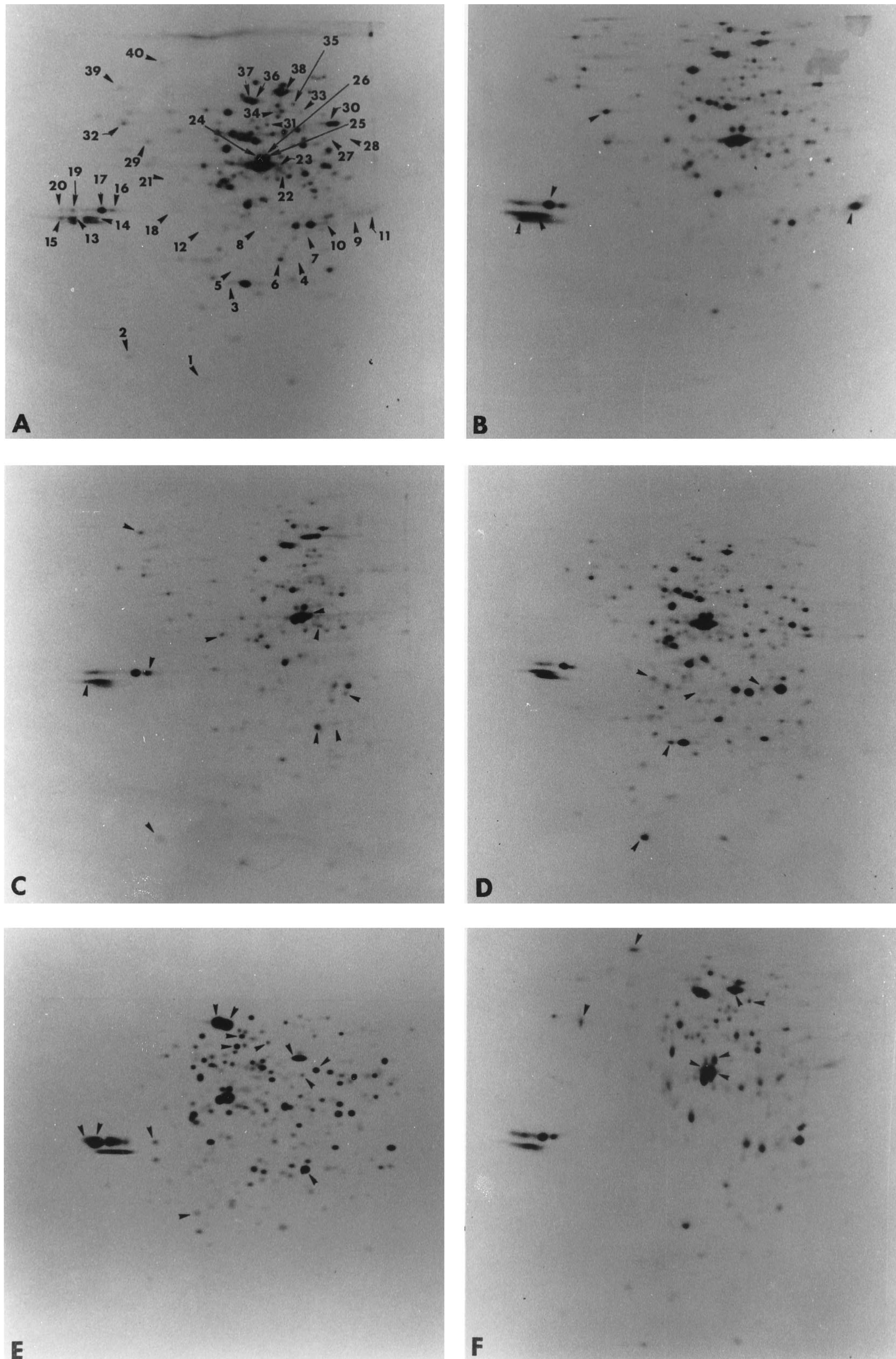

FIG. 2. Cold-induced proteins in *V. vulnificus*. Protein samples were radiolabeled, extracted, and then resolved by two-dimensional polyacrylamide gel electrophoresis. Densitometry was used to identify proteins induced by a factor of 2 or more in response to a temperature downshift from 23 to 13°C. Arrows (A) mark fully induced proteins. Images of gels were aligned and stretched to fit a common standard with Pharmacia Imagemaster software. (A) Pattern of protein synthesis at 23°C. (B through F) Patterns of protein synthesis at 15 and 30 min and 1, 2, and 4 h, respectively, after shift to 13°C.

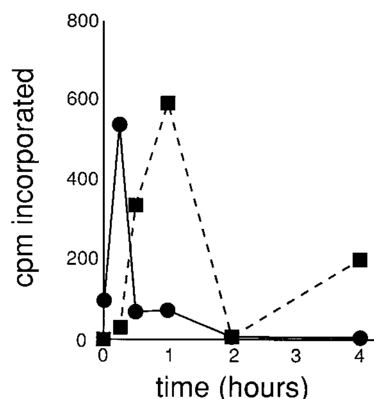

FIG. 3. Major patterns of cold stress protein induction in *V. vulnificus*. Induction rates of the class I major cold shock protein 1005 (Fig. 2, arrowhead 1) (squares) and of a class II protein (Fig. 2, arrowhead 29) (circles) are shown. See text for descriptions of classes I, II, and III.

Induced proteins fell into three classes. For the 12 class I proteins, induction peaked, declined, and then rose again as the cells resumed growth at the new temperature (Fig. 3). Induction of the 26 class II proteins rose to a maximum and then declined. The two class III proteins were induced only after 4 h in the cold. We speculate that the first class represents titratable factors that are synthesized to high levels and then maintained. The second group may represent true survival factors, needed only as cells adjust to growth at new lower rates.

One protein (Fig. 2A, arrowhead 1) was designated protein 1005 by computer-generated sample spot number. This factor was strikingly induced by temperature downshift. Although protein 1005 was absent at room temperature, its expression increased by a factor of 35 within an hour of cold shift. Although this protein's time of induction and absence during normal growth are reminiscent of CspA, its apparent molecular mass (16.4 kDa) and apparent pI (5.02) are very different from those (7.4 kDa and 5.9, respectively) of CspA.

More profound cold stress has been identified as a signal for *V. vulnificus* to enter a nongrowing state. Prestarvation of cells protects the cells from cold stress (9). Recent studies in this laboratory (6) have examined the proteins induced by starvation. None of the major starvation proteins coincide with the cold stress proteins identified in this report. Ongoing studies for understanding the interactions between these two classes of stress response are under way.

This study was supported by a grant (NA36FD0271) from the National Marine Fisheries Division of NOAA.

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